PHYTOCHEMICALEVALUATIONAND*IN-VITRO*ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTI-DIABETIC ACTIVITY ASSESSMENT OF *LINUM USITATISSIMUM* L. SEED EXTRACT

ABSTRACT

Objectives: *Linum usitatisimum*(L.), commonly known as flaxseed, is a rich source of differenttypesofphytochemicalsandpossessesnutritiveandtherapeuticvalue.Thestudywas carried out to evaluate phytochemicals, antioxidant, anti-inflammatory and anti-diabetic activity of flaxseed extract, targeting its potential use in managing oxidative stress,inflammation, and metabolic disorders.

Methods:Flaxseedpowderwasdefattedusinghexane,anddefattedpowderwasthenextracted by maceration with 80% ethanol. Phytochemical evaluation was performed using standard screening methods and Fourier transform infrared (FTIR) spectroscopy. The total phenolic content was determined using the Folin-ciocalteu method. The antioxidant activity of the extract was determined by free radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The anti-inflammatory activity was assessed by human RBC membrane stabilization assay, which mimics lysosomal membrane stabilization. The anti-diabeticactivityofextractwasdeterminedbyα-amylaseandα-glucosidaseenzymeinhibition assaysto assess the extract's ability to regulate carbohydrate metabolism.

Results: Qualitative phytochemical analysis of ethanolic extract confirmed the presence of alkaloids, phenols, flavonoids, tannins, phytosterols, carbohydrates, and glycosides. FTIR analysis of the extract demonstrated **the** presence of the compounds phenols, flavonoids, and tannins. The total phenolic content was found to be 90.98 mg gallic acid equivalent / g of dry extract,indicativeofitsantioxidantcapacity.Themedianinhibitoryconcentration(**IC_{50}**)value of ethanol extract for antioxidant activity was found to be 297.39 µg/ml. The extractdemonstrated dose-dependent inhibition of RBC hemolysis, with an **IC_{50}**value of 197.312µg/ml, highlighting its anti-inflammatory efficacy. The **IC_{50}**value of extract against alpha- amylase was found to be 8.54 mg/ml. The plant extract showed an inhibition of 31.52 % at concentration of 10 mg/ml against alpha-glucosidase.

Conclusion:Theseresultssuggestthatflaxseedhaspromisingantioxidant,anti-inflammatory, andantidiabeticproperties,makingitavaluablecandidateforfurtherresearchandtherapeutic applications.

Keywords:*LinumUsitatissimum*L.,Ethanolicextract,Phytochemicalscreening,Antioxidant activity, Anti-Inflammatory activity, Anti-diabetic activity.

1. INTRODUCTION

"Herbs and spices have been used to treat various ailments since ancient times. Modern medicinesarediscoveredanddevelopedusingphytochemicalsthatareextractedfromplants"[1]. "It is estimated that more than 25% of modern medicines are derived directly or indirectly from plants" [2,3]. "Due to their pharmacological activity, low toxicity, and cost-effectiveness when compared to synthetic drugs, medicinal plants are becoming more and more popular worldwide as a viable option for treating chronic illnesses. Diabetes mellitus is a growing globalhealthconcern,affectingover536millionpeopleworldwideandprojectedtoaffect643 million by 2030 and 783 million by 2045" [4]. It is a chronic disease characterized by hyperglycemia and carbohydrate, protein, and fat metabolism disturbances caused either due to the insufficient production of insulin or the body's inability to use insulin effectively. Medicinalplantsremainvitalfordiabetestreatment,especiallyindevelopingcountrieswhere the majority of people lack access to modern medicines and have limited financial resources. Anti-diabeticmedicationslikeacarboseandvoglibosecomewithgastrointestinalside effects, highlighting the need for safer and more effective alternatives. One promising therapeutic approach involves inhibiting α-amylase and α-glucosidase, two enzymes responsible for carbohydrate metabolism, for managing blood glucose levels in individuals with type 2 diabetes. Inflammation is a biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. However, chronic inflammation can lead to tissue damage. Conventional anti-inflammatory drugs, like steroids and NSAIDs, have side effects, whichhasledtogrowinginterestinmedicinalplantswithanti-inflammatoryproperties,which may not only suppress inflammation but also address various diseases where inflammation exacerbates the condition.

"*Linumusitatissimum*,commonlyknownasflaxseedorlinseed,isavaluableherbbelongingto the family Linaeceae.It is an annual herb and may grow to heights of 60-120 cm. The fruits arecapsulararthatconsistoffivecellswithapairofseedsineach.Theseedsareovate,flattened, and obliquely pointed at one end, about 4-6 mm long and 2-2.5 mm broad. Flaxseed consists of bioactive compounds like alkaloids, flavonoids, polyphenols, tannins, lignans (notably secoisolariciresinoldiglucoside), omega-3 fatty acids, alpha-linolenic acid, dietary fibers, sterols, protein, and glycosides. These compounds have demonstrated a wide range of biologicalandpharmacologicalactivityagainstvariouschronicdiseasessuchascardiovascular disorders,cancer,arthritis,etc"[5-7]."Antioxidant,anti-diabetic,anticancer,antimicrobial,anti-obesity,anti-inflammatoryeffectsofflaxseedextracthavealso beenreportedonvariousanimal

Models"[8–13]. Flaxseed has gathered significant attention in recent years as a potential nutraceutical,duetoitshighcontentofomega-3fattyacids,alpha-linolenicacid,dietaryfiber, and lignans.

Despitethewidespreadrecognitionofflaxseed'snutritionalandmedicinalproperties,thereare few in-depth studies on their phytochemical, antidiabetic, and anti-inflammatory potential. Thisgapisparticularlyimporttoaddressgiventherisingprevalenceofvarioushealthissues and illnesses, many of which can be linked to lifestyle factors such as diet and obesity. Although many research studies have been carried out on flaxseed; particularly in countries like Nepal, where altitude and climatic variations could influence its properties, are yet to be explored. This study aims to investigate and evaluate the phytochemical constituents of flaxseed and to assess its various activities, including antioxidant, antidiabetic, and anti- inflammatory properties. By providing scientific evidence supporting its traditional uses, this research seeks to explore flaxseed's potential as a source of novel therapeutic agents.

2. MATERIALANDMETHODS

2.1 Plantmaterial

*Linumusitatisiumum*seedswereboughtfromlocalmarketinAsan,Kathmandu.Theflaxseed wasthenwashedwithwatertoremoveanydirtifpresentandlefttoshadedryfor3days. The dry seeds were then grinded to fine powder using a grinder. The powder was then passedthrough mesh number 22. Finally, the sieved powder was stored in air-tight container at 25^0C for further use. The plant material was authenticated from National Herbarium and Plant Laboratories, Godavari.

2.2 Extraction

About 50 gm of flaxseed powder was defatted by macerating the powder using 300 ml of n-hexane (1:6 w/v) for around 16 hours. The defatted flaxseed powder was then filtered using Whatman filter paper no. 41 and left to air dry. About 35 gm of the air dried powder was weighed and macerated in 350 ml of 80% ethanol for around 48 hours. The extract was then filtered and concentrated in a rotary evaporator. The concentrated extract was then stored in the refrigerator with proper labeling and used for further research purpose.

2.3 Determinationofphysiochemical parameters

2.3.1 Flow property

2.3.1.1 Tappedandbulkdensity

- For bulk density, 30gm of the powder was taken and kept in a graduated measuring cylinder. The volume that it covered was noted and the bulk densitywas calculated using formula:

  Bulkdensity=(weight of thepowder taken ÷ volume covered bypowder).

- Fortappeddensity,themeasuringcylinderwasthentappedonthesurfaceuntilthe volume that it occupied was constant.

  Tappeddensity=(weightof thepowdertaken÷ volumecoveredafter tapping)

2.3.1.2 Hausner ratio

HR=Tapped density÷Bulk density

2.3.1.3 Carr'sindex

C.I=(Tapped Density-Bulk density)÷Bulk density×100%

2.3.2 Lossondrying

Thehotairovenwasswitchedonandlefttoheatup.ThePetriplatewascleanedandleftinside the hot air oven for about 30 minutes. About 10 gm of flaxseed powder was weighed and put inthepetriplateandinsidethehotairovenat105□foraboutanhour.Anhourlater,thePetri platewastakenoutfromthehotairoven,lefttocoolindesiccatorandtheweightofthepowder was taken.

LOD=(weightloss÷ weightofsample) ×100

2.3.3 Swellingindex

Itisthevolumeinmltakenupbyswellingof1gmofplantmaterialunderspecifiedcondition. 1gmofplantmaterialwastakenin50mlgraduatedmeasuringcylinderand 25mlofdistilled water was added to it. The mixture was shaken thoroughly every 10 minutes for an hour and was allowed to stand for 3 hours. The volume occupied with the plant material was noted.

2.3.4 Extractivevalue

It is used for the estimation of constituents extracted with solvent usedfor extraction. 100 gm offlaxseedwasdefattedusing600mlofhexane.Thedefattedpowderwasair-driedandabout 72.5gmofdriedpowder wasmaceratedin720mlethanolfor2days.Theextractwasfiltered and concentrated using rotary evaporator. Finally yield value was calculated using the following formula:

%yield=(weightofdriedextract÷weightofdriedplantsample)×100%

2.4 Phytochemicalscreening

The qualitative phytochemical screening for the various constituents present in plant extract wasperformedaccordingtothestandardmethoddescribedbyJunaidR.ShaikhandMKPatil. [14]

2.5 Totalphenoliccontentassay

Thetotalphenoliccontentintheethanolextractwasdeterminedbytheolin-Ciocalteureagent method as described by Singleton and Rossi [15] with minor modifications. 1 mL of plant extractwastreatedwith5mLofFolin-Ciocalteureagentandallowedtostandfor5minutes.4 mL of sodium carbonate solution was added to the above mixture. The reaction mixture was allowedtostandinthedarkforabout1hour,andtheabsorbancewasmeasuredat765nm usingmethanolasablank.Gallicacidwasusedasastandard.Thecalibrationcurvewasplotted with a standard of solution of gallic acid in the range of 25-500 µg/ml.The total phenolic content in the sample was determined as milligrams of gallic acid equivalent by using the following equation:

$$TPC = C \times (V \div M)$$

WhereC=concentrationofGallicacidfromcurve(mg/mL) V = volume of extract (mL)

M=weightofplantextract(gm)

2.6 Totalflavonoidcontent

Thetotalflavonoidcontentintheextractwasestimatedbythealuminumchloridecolorimetric method.200µlofaluminumchloridesolution(10%w/v)wasaddedto1mlofextractand200µl of potassium acetate (0.1 M), and finally, 4 ml of distilled water was added to the mixture. Aftervortexmixing,thesolutionwasstoredindarkfor45minutes.Finally,theabsorbanceof the mixture was measured at 415 nm. Quercetin was used as a standard. The total flavonoid content in the sample was calculated as milligrams of quercetin equivalent by using the following equation:

$$TFC = C \times (V \div M)$$

WhereC=concentrationofquercetinfromcurve(µg/mL) V = volume of extract (ml)

M=weightofplantextract (g)

2.7 FTIRanalysis

The dried ethanol extract of flaxseed was subjected to FTIR analysis to detect various compounds and functional groups. The IR spectra were measured using an FTIR spectrophotometer (IRPrestige-21 Shimadzu). The spectra were captured at a resolution of 1 cm^{-1} in the range of 4500–500 cm^{-1}.

2.8 Antioxidantactivity:

The antioxidant activity of plant extract was determined by 1,1- diphenyl-2-picryl hydrazyl radical(DPPH)scavengingmethodaccordingtoAlacheretal.[16].1mMDPPHsolutionwas prepared, and 150 µl of this solution was added to 50 µl of extract at different concentrations (15.625,31.25,62.5,125,250,500µg/ml).Thenthemixturewasallowedtostandin thedark at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a microplate reader. Ascorbic acid was used as a standard. All the tests were carried out in triplicate. Then finally a calibration curve was plotted.

Thepercentageofradicalscavengingactivitywasdeterminedusingtheformulabelow:

Percentage scavenging = $(A_o-A_T)/A_T \times 100$ %

Where,A_o=Absorbance ofcontrol(DPPH only).

A_T=Absorbance of sample.

IC_{50}valueisdefinedastheeffectiveconcentrationofthesamplerequiredtoscavenge50%of the DPPH free radicals. IC_{50}values were determined by plotting the concentration of extract versus the corresponding scavengingeffect. Lower the IC_{50}value, the higher the antioxidant activity.

2.9 *In–vitro*anti-inflammatoryactivity

The anti-inflammatory activity of the extract was studied using a human RBC membrane stabilization assay[17]. The principle behind this method is the stabilization of human red blood cell membrane by hypo tonicity-induced membrane lysis.This assay is based on the principle that the erythrocyte membrane resembles the lysosomal membrane, and its stabilization can be an indicator of the anti-inflammatory activity of the substance being treated.

A blood sample was taken from a healthy volunteer student who had not taken any NSAIDs fortwoweeksanddistributedintotubescontainingethylenediaminetetraaceticacid(EDTA) (1%) solution using a 3 ml syringe. The blood solution was centrifuged at 3000 rpm for 10minutes,andserumwasdiscarded.Thebloodsampleswеremixedwiththesameamountof

Alsever's solution, (containing, 0.8 gm sodium citrate, 2 gm of dextrose, 0.05 gm citric acid, and 0.42 gm of sodium chloride, dissolved in 100 mL of distilled water) and centrifuged at 3000rpmfor10minutes.Thenthesupernatantwasdiscarded,andthepelletwaswashedagain threetimeswithisotonicsaline.A10%v/vsuspensionofRBCwasmadeinnormalsalineand kept at 4°C undisturbed before use.

Different concentrations (62.5, 125, 250, 500, 1000 µg/mL) of extracts were prepared. Test sampleswerepreparedusing1mlphosphatebuffer,2mlhypotonicsaline,0.5mloftheplant extract,and0.5mlofRBCsuspension.Theassaymixtureswerethenincubatedat37°Cfor30 minutes.Afterincubation,theassaymixtureswerecentrifugedat3000rpmfor10minutesand carefully handled without disturbing the pellet. Then the absorbance of the supernatant was measuredat560nm.Thewholetestprocedurewasdoneintriplicates.Diclofenacsodiumwas used as a standard and the control sample was prepared by omitting extracts.

%Protection(stabilization)oftheRBCmembranewasmeasuredbyusingfollowingformula:

%Protection =100 – [Absorbanceof thesample÷Absorbanceofthecontrol]×100.

2.10 *In-vitro*antidiabeticactivity:

1. α-Glucosidaseinhibitoryassay

   α-Glucosidase inhibition was measured using p-nitrophenyl-α-D-glucopyranoside (p-NPG)as the substrate [18].Different concentrations of linseed extract, rangingfrom 1 to 10mg/mL, were prepared (dilution in phosphate buffer). 40 µl of extract was incubated with the solution containing 20 µl of α-glucosidase (1 U/mL) for 30 min at 37 °C. 40 µl of 2.5 mM para-nitrophenyl-α-D-glucopyranoside (PNPG) in 0.1 M potassium phosphate buffer was added to initiate the reaction, and the mixture was furtherincubatedfor15 minutes.Finally, theabsorbance ofthemixturewas recorded at 405 nm. Acarbose was utilized as the positive control, and each concentration was tested in triplicate. The results were presented as % inhibition of enzyme activity and calculated using the following formula:

   $$\%\text{Inhibition}=[(X_A-X_B)/X_A] \times 100.$$

   X_A is the absorbance of the control (100% enzyme activity), and $X_B$is the absorbance of the sample.

2. α-Amylaseinhibitoryassay

   α-Amylase inhibition was measured using 2-Chloro-4-Nitrophenyl-α-Maltotrioside($CNPG_3$)asthesubstrate.Differentconcentrationsoflinseedextract,rangingf rom1to 10mg/mL,wereprepared(dilutioninphosphatebuffer).20µlofextractwas

incubatedwiththesolutioncontaining80µlofα-amylase(2U/mL)for30minat37 °C. 100 µl of 1 mM $CNPG_3$in 0.1 M potassium phosphate buffer (6.9) was added to initiate the reaction, and the mixture was further incubated for 15 min. Finally, the absorbance of the mixture was recorded at 405 nm. Acarbose was utilized as the positive control, and each concentration was tested in triplicate. The control was preparedbyaddingdeionizedwaterinsteadofplantextract.Theresultswerepresented as % inhibition of enzyme activity and calculated using the following formula:

$$\% \text{ Inhibition} = [(X_A - X_B)/X_A] \times 100.$$

X_A is the absorbance of the control (100% enzyme activity), and $X_B$is the absorbance of the sample.

3. RESULTS

3.1 Physiochemicalparameters

3.1.1 Flow property

3.1.1.1 Bulkdensity=weight ofthe powdertaken ÷volumecovered bypowder

=30÷70.7 = 0.424 gm/cm³

3.1.1.2 Tappeddensity=weight ofthepowder taken÷ volume covered after tapping

=30÷57 =0.526gm/cm³

3.1.2 Hausner ratio

HR=Tappeddensity÷Bulkdensity

=0.526÷0.424

=1.24

3.1.3 Carr'sindex

C.I=(Tapped Density-Bulk density)÷Bulk density×100%

=[(0.526-0.424)÷0.424] ×100%

=24.06 %

3.1.4 Lossondrying

LOD=(weightloss÷ weightofsample) ×100

=(10.001 -9.066)÷10.001×100 %

=9.349 %

3.1.2 Swellingindex=[(0.7-0.4) ÷0.4] ×100%=75 %

3.1.3 Extractivevalue

%yield=(weightofdriedextract÷weightofdriedplantsample)×100%

=(1.863÷72.5)×100 %=2.569%.

3.2Phytochemicalscreening analysis

Theresultsofphytochemicalscreeningofflaxseedareshownbelow: Table 1:

Phytochemical screening of flaxseed ethanol extract

S.N.	Chemical constituents	Phytochemical test	Observation	Result
1.	**Flavonoids**	Leadacetatetest Shinoda test $FeCl_3$ test Conc.H_2SO_4test	Yellow precipitate Crimson red color Green precipitate Lightorange color`	+ve +ve +ve +ve
2.	Phenols	$FeCl_3$test $K_2Cr_2O_7$ test Iodinetest	Darkgreenprecipitate Light dark color Redcolor	+ve +ve +ve
3.	Alkaloids	Wagnerstest Hagers test Mayerstest	White precipitate Brown precipitate Nochangeincolor	+ve +ve -ve
4.	Phytosterols	Salkowskitest	Ringatthejunctionandredcolor in lower layer	+ve
5.	Carbohydrates	Molischtest	Violetring	+ve
6.	Cardiac glycosides	KellerKillanitest	Bluishblack color	+ve
7.	Saponin	Frothtest	Frothingpresent	+ve

3.3 Totalphenoliccontentanalysis:

Thetotalphenoliccontentwasdetermined asamilligramofGallicacidequivalentusingthe calibrationcurveofGallicacid.Theabsorbanceofeachsolutionwasmeasuredandnotedas follows:

Table2:AbsorbanceofGallic acid

Concentration(µg/ml)	Average absorbance
25	0.115
50	0.179
100	0.239
250	0.461
500	0.733

Figure1:Calibrationcurveofgallicacidfortotalphenoliccontentdetermination

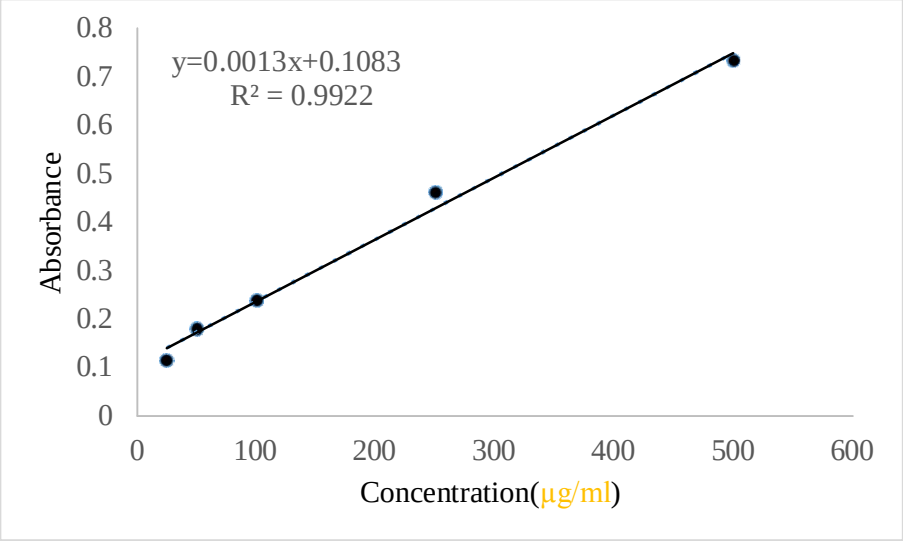


Table3: Totalphenolic contentin ethanolextract of flaxseed

Sample concentration	**Average absorbance**	**Concentration mg/ml**	**TPCasGAE (mg/g)**
48 mg/ml	0.474	0.281	90.98

3.4 Totalflavonoidcontent analysis:

TFCisexpressedasmgofquercetinequivalentspergramofdriedsample.Theabsorbanceof each solution was measured and noted as follows:

Table4:Absorbanceof quercetin

Concentration(µg/ml)	Average
1	0.07503
5	0.1038
10	0.195
20	0.2645
40	0.3968

Figure2: Calibrationcurveof quercetinfortotalflavonoid content determination

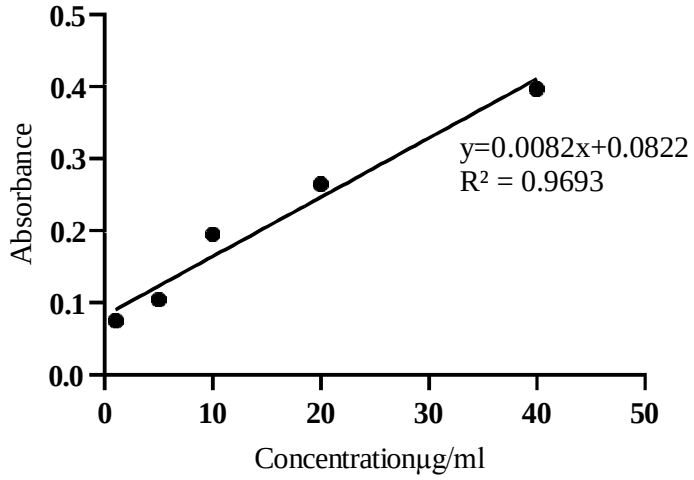


Table5: Totalflavonoid contentin ethanolextract of flaxseed

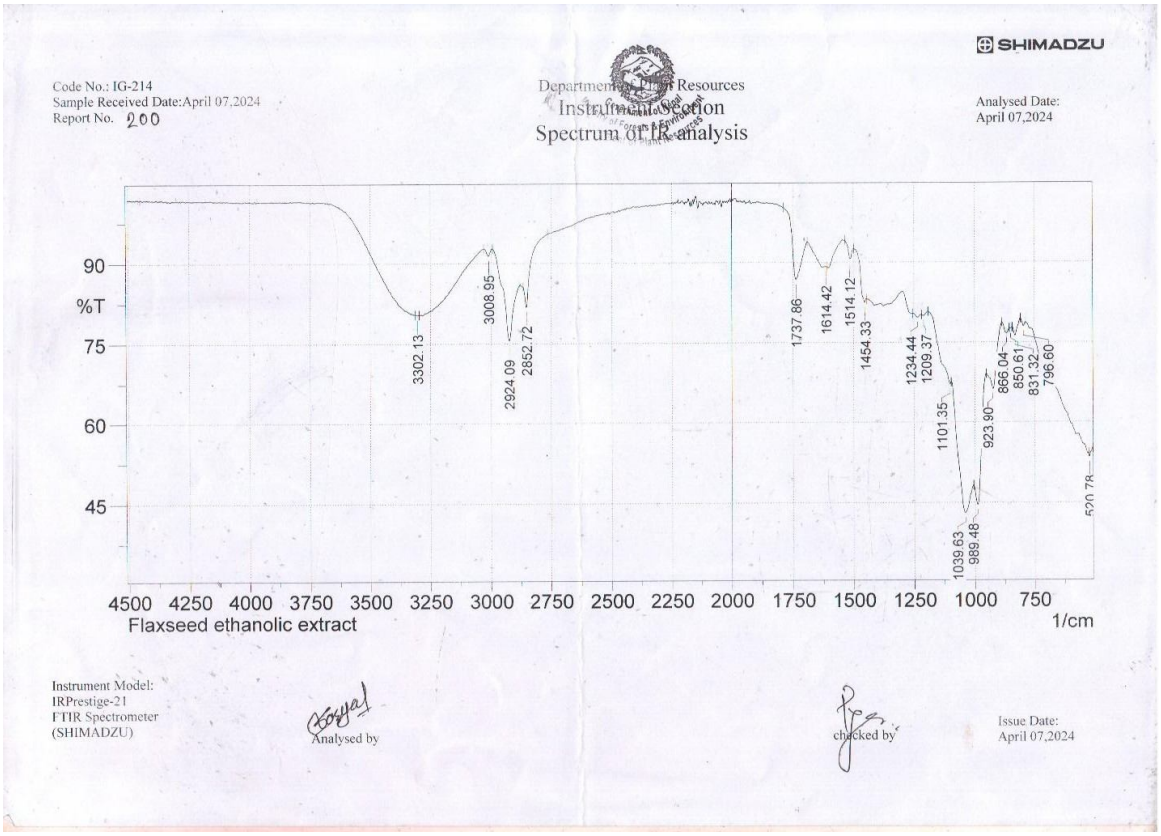
Sample concentration	Average absorbance	Concentration mg/ml	TFCasQE(mg/g)
20 mg/ml	0.2796	0.024073	1.20

3.5 FTIRanalysis

FTIR spectra of the flaxseed extract revealed a variety of distinctive IR absorption peaks at wavenumbersin the aromaticand fingerprint regions ofthe IR band. The broad peak at 3400 cm^{-1}results from -OH groups (alcohol and phenols), and the 3008 cm^{-1}peak from aromatic C-Hbonds.Theabsorptionbandsat2924and2852canberelatedto–CHstretchingvibrations. The1737cm^{-1}wave-numberpeaksresultfromthepresenceofC=O(carbonylgroups),

functional groups arising from flavonoids and tannin derivatives. 1039 cm^{-1}, 1101 cm^{-1}, 989 cm^{-1}wave-number peaks result from C-O stretching vibrations of alcohols, carboxylic acids, esters, or ethers of biomolecules found in the flaxseed. The absorption peaks at 1614 cm^{-1} could be assigned to C=C stretching of alkene or aromatic compounds. The absorption peaks at 1514 cm^{-1} could be assigned for the presence of N–O stretching in nitro. The small peaks between890-796cm^{-1}correspondtochloroalkane(C-Cl)and=C-HbendingaromaticC-Hout of plane bend.

Figure3:FTIRspectraofethanolextract



3.6 Antioxidantanalysis:

ThepercentageofDPPHradicalscavengingincreasedwithhigherconcentrationsofthe extract, demonstrating a dose-dependent effect as shown in following table:

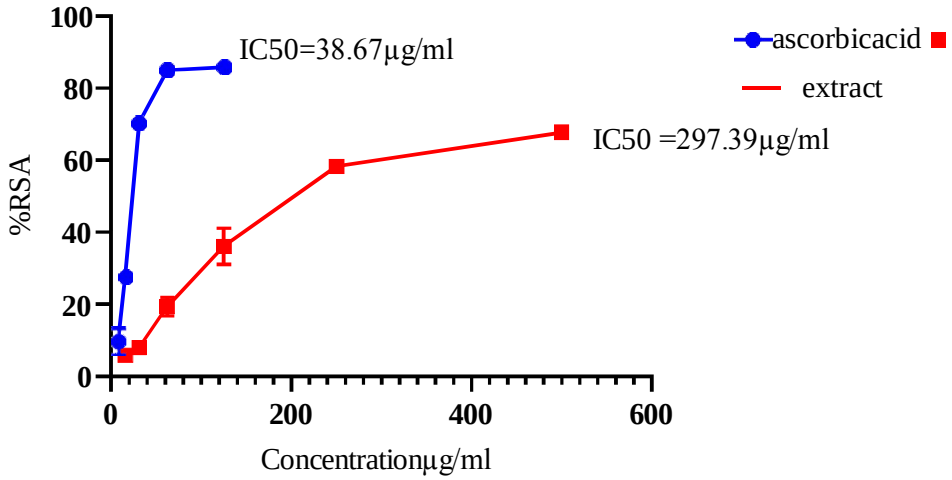
Table6: DPPH scavengingactivityofethanol extract

Concentration µg/ml	%RSA (mean±S.E.M)
15.625	6.707 ± 1.59
31.25	8.076 ± 0.79
62.5	19.37 ± 1.51
125	36.13 ± 2.91
250	58.31 ± 0.77
500	67.69 ± 1.07

Table7: DPPHscavengingactivityofascorbic acid

Concentration mcg/ml	%RSA (mean±S.E.M)
7.8125	2.737 ± 0.59
15.625	9.034 ± 1.48
31.25	29.08 ± 1.85
62.5	70.294 ± 1.00
125	84.94 ± 0.18

Figure4: DPPHscavengingactivityofextractandascorbic acid



3.7 *In-vitro* anti-inflammatory analysis:

The percentage protection of RBC was relatively lower in samples treated with the extract compared to diclofenac as shown in following table:

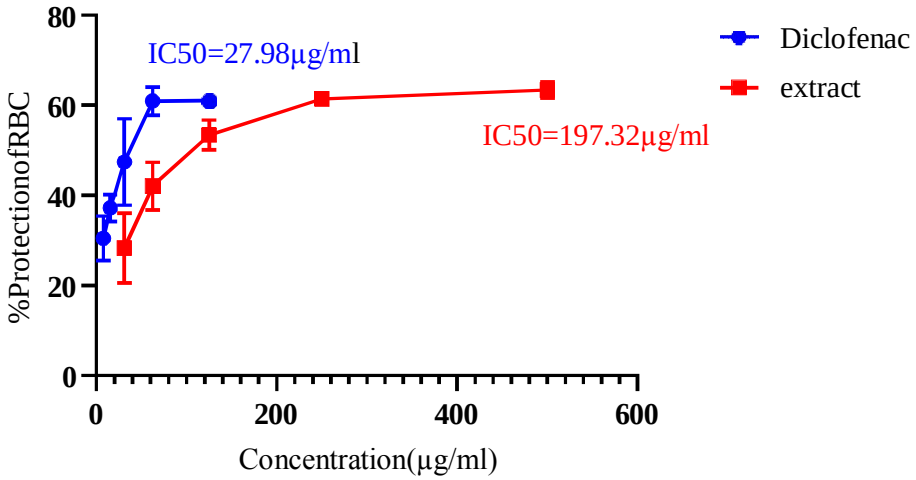
Table8:AntiInflammatoryActivityofdiclofenacbyHRBC Method

Concentration (mcg/ml)	**%Protection (mean ± S.E.M)**
7.8125	30.53 ± 2.03
15.625	37.21 ± 1.23
31.25	47.43 ± 3.93
62.5	60.93 ± 1.30
125	61.13 ± 0.34
250	61.43 ± 0.61
500	63.53 ± 1.33

Table9:AntiInflammatoryActivityof extractbyHRBC Method

Concentration(mcg/ml)	%Protection (mean±S.E.M)
31.25	28.395 ± 3.19
62.5	42.121 ± 2.15
125	53.421 ± 1.38
250	61.456 ± 0.64
500	63.465 ± 0.74

Figure5: AntiInflammatoryActivityofextractand diclofenacbyHRBCMethod



3.8 Antidiabetic activity:

3.8.1 Alpha– glucosidaseassay:

Theinhibitoryactivityoftheextractandacarboseagainstα-glucosidasewere measured and presented in the following table:

Table10: α-glucosidaseinhibitoryactivityof flaxseed extract

Concentration mg/ml	%inhibition (mean±SEM)
1	7.903 ± 0.28
2	9.778 ± 1.78
4	13.77 ± 2.88
6	16.40 ± 2.28
8	25.71 ± 3.48
10	31.52 ± 3.98

Table 11: α-glucosidase inhibitoryactivityofacarbose

Concentration mcg/ml	%inhibition (mean±SEM)
100	8.538 ± 0.59
200	20.689 ± 0.49
300	48.440 ± 0.32
350	54.187 ± 0.28
400	64.203 ± 0.59
450	77.504 ± 1.07

Figure6: α-glucosidaseinhibitoryactivityof flaxseedextract

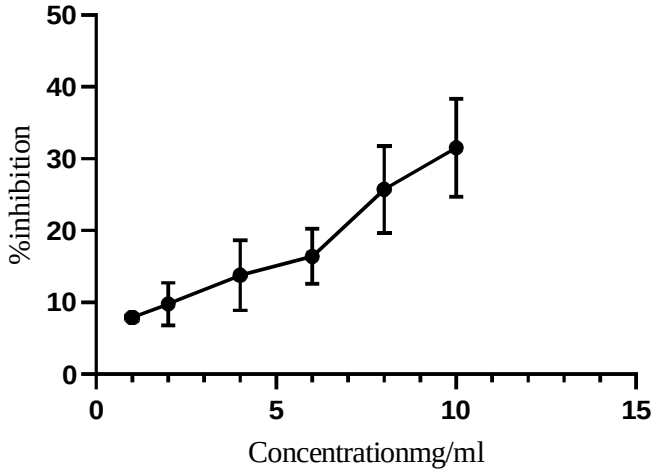
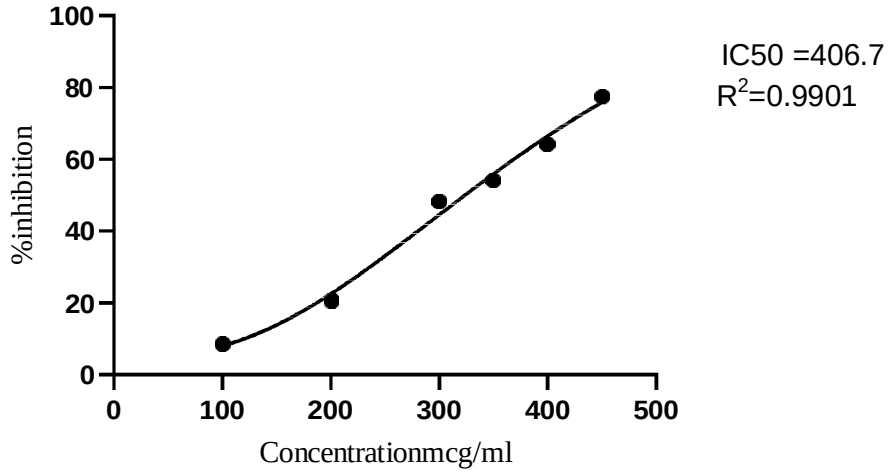


Figure7:α-glucosidaseinhibitoryactivityofacarbose



3.8.2 Alpha-amylaseassay:

Theinhibitoryactivityoftheextractandacarboseagainstα-amylaseweremeasured and presented in following table:

Table12: α-amylaseinhibitoryactivityof flaxseed extract

Concentration mg/ml	%inhibition (mean±SEM)
1	2.954545455
2	15.22727273
4	20.07575758
6	39.92424242
8	45.22727273
10	58.56060606

Table13: α-amylaseinhibitoryactivityofacarbose

Concentration mcg/ml	%inhibition (mean±SEM)
100	67.68 ± 0.50
200	70.30 ± 1.46
300	83.57 ± 0.11
350	89.59 ± 0.10
400	92.65 ± 0.13
450	93.71 ± 0.03

Figure9:α-amylaseinhibitoryactivityofflaxseedextract

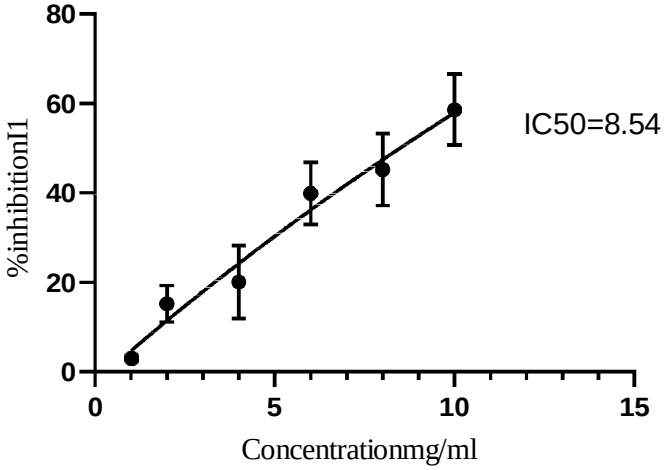
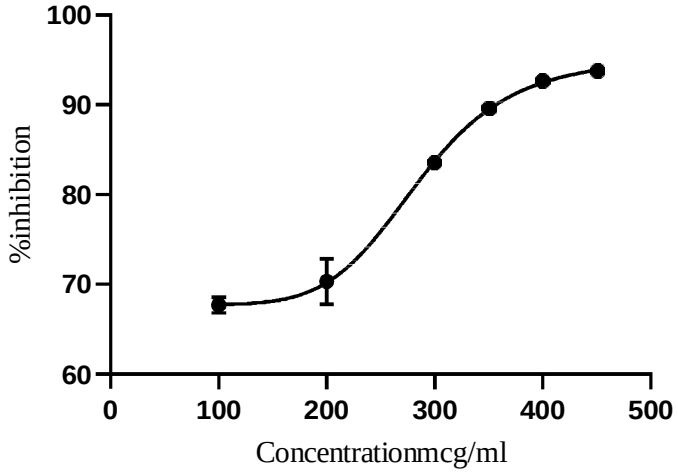


Figure10:α-amylaseinhibitoryactivityofacarbose



4. DISCUSSION

Physicochemicalevaluationisessentialfordetectingadulterationormishandlingsinceadrug's efficacy depends on its physical and chemical properties. This assessment ensures the authenticityand purityofthematerial.Thebulkdensityandtappeddensitywerefoundtobe 0.424 and 0.526 gm/cm^3, respectively. Carr's index was found to be 24.60%, suggesting moderate flowability. The loss on dryingwas found to be 9.34%. This value is slightlyhigher than the value reported by the Canadian Grain Commission [19], where moisture content was found to be 7.7%, 7.2% reported by Eggie [20] , and 7.72% as reported by Khan [21] . The swellingindexof75%indicatesthatthevolumeofthematerialincreasedby75%ofitsoriginal size where it absorbs the particular solvent. The extractive value was found to be 2.5%.

The phytochemical analysis of L. usitatissimum ethanol extract showed the presence of flavonoids, phenolic compounds, glycosides, alkaloids, terpenoids, tannins, phytosterols, proteins, and carbohydrates. Thesephytochemicals areresponsible for the versatile medicinal properties of the extract like antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activity. Amin and Thakur [22] reported "that phytochemical analysis of ethanol extract of flaxseeds contained tannins, flavonoids, terpenoids, phenols, proteins, and amino acids while it did notcontain saponins, sterols, and glycosides. FTIR analysis of the extract demonstrated the compounds like phenols, flavonoids and tannins".

The total phenolic content in the flaxseed was found to be 90.98 mg gallic acid equivalent / g ofdryextract.Thevalue ofTPCwasfoundtobehigherincomparisontothestudyperformed byHanaa M. H. et al. (15.5mg/gGAE) [11] andAlachaheet al. [16](47.2mg/gm GAE).Ourdataisinagreementwiththestudieswhichdemonstratedthat70%ethanoland70%methanolextracting solvents were more effective isolatingphenolic compounds from different plantmaterials [23]. Sultana et al.[24], also found that 80% aqueous ethanol showed the besteffectiveness in extracting phenolic components from barks of some plants.Bonoli et al. [25] reportedthatmaximumamountofphenoliccompoundswasextractedfrombarleyflourwhen aqueous ethanol and acetone were applied as extractants."Phenolic compounds have a diverse set of physiological benefits that include reducing inflammation, protecting against oxidative stress, anti-cancer, cardio-protective activity, and altering gene expression" [26,27].The total flavonoid content in the flaxseed was found to be 1.20 mg quercetin equivalent / g of dry extract.TheTFCvalueweobtainedwassimilartotheonereportedbyHanaaM.H.etal[15], where it was found to be 2.5 mg/gm QE.

DPPH method is one of the reliable and widely used methods for evaluating antioxidant activity. The extent of reaction mainly depends on the hydro gen donating ability of the antioxidants whichispredominantlygovernedby theirstructureanddegreeofhydroxylation [28].Theantioxidantpotentialshows an inverserelationshiptothe IC_{50}value.The IC_{50}value oftheextractwasfoundtobe297.306µg/ml.ThisresultisinlinewithAminT.andThakur M. [21], where the IC_{50}value of the ethanol extract of flaxseeds was 256.313 µg/ml. The antioxidant activity may be due to the presence of phenols, lignans, and flavonoids. The findings suggest that incorporating flaxseed extracts into functional foods may enhance their health benefits and contribute to the prevention of oxidative stress-related diseases.

The anti-inflammatory activity was studied by using an RBC membrane stabilization assay. TheresultsdemonstratedthattheextracteffectivelystabilizedtheRBCmembranes,indicating anti-inflammatoryactivity. The IC_{50}valueoftheextractwasfoundtobe 197.312 µg/ml. The resultsalsorepresentthatextractcandose-dependentlyinhibitRBChemolysis.Similarresults were obtained from the study of Alawlaqi et al. [18] and Vidyasabbaniet al.[30],where hemolysis inhibition (%) increased with increasing linseed extract concentration in the range of100upto1000µg/ml.Thesefindingssupportpreviousresearchontheroleofplantextracts in mitigating inflammation.

“Alpha-amylase and alpha-glucosidase are the carbohydrate-metabolizing enzymes. Alpha-amylase catalyzes the breakdown of polysaccharides like starch into maltose and dextrin, whereas alpha-glucosidase further breaks down these oligosaccharides into monosaccharides like glucose. Targeting and suppressingenzyme is one of the potential methods of avoidingincreased postprandial blood glucose”[30,31].“There was a dose-dependent increase in the percentageinhibitoryactivityoftheextractagainstα-amylaseandα-glucosidaseenzymes.The plant extract showed a percentage inhibition of 2.95% and 58.56% against α-amylase at concentrations of 1 mg/ml and 10 mg/ml, respectively. The IC_{50}value of the extract against amylase was found to be 8.54 mg/ml. This value is higher than the IC_{50} value of 300 mcg/ml as reported by Mechchate et al” [32] . This may be due to the use of crude extract rather than isolated compounds. The plant extract showed an inhibition of 31.52% at a 10 mg/ml concentration against alpha-glucosidase. Mohamed M. Alawlaqi et al. [18] reported the IC_{50}value of methanol extract of linseed against alpha-glucosidase as 177.75 µg/ml. The predominance of alpha-amylase activity suggests that flaxseed may be more effective for breakingdownlargerpolysaccharidesratherthanaidinginthefinalstepsofcarbohydrate

digestion.Theinhibitoryactivitymaybeduetothepresenceofphenols,lignans,carbohydrates and dietary fibers.

5. CONCLUSION

Flaxseed(*LinumusitatissimumL.*)isamulti-purposecrop,anditsconsumptionhassignificant healthbenefits.Fromtheresultofthisstudy,itcanbeconcludedthatvariousphytochemicals, including alkaloids, phenols, flavonoids, saponins, proteins, glycosides, and phytosterols, are present in flaxseed which are responsible for various activities of extract. The present study demonstrated that the flaxseed extract exhibits potential antioxidant, anti-inflammatory, and antidiabeticactivityinan *in-vitro*model.So,furtherisolation,purificationand *in-vivo*studies are essential to explore its full therapeutic potential.

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